Interaction of Imatinib with Human Organic Ion Carriers

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Abstract

Purpose: The activity of imatinib in leukemia has recently been linked with the expression of the organic cation transporter 1 (OCT1) gene SLC22A1. Here, we characterized the contribution of solute carriers to imatinib transport in an effort to further understand mechanisms involved in the intracellular uptake and retention (IUR) of the drug.

Experimental Design: IUR of [3H]imatinib was studied in Xenopus laevis oocytes and HEK293 cells expressing OATP1A2, OATP1B1, OATP1B3, OCT1-3, OCTN1-2, or OAT1-3. Gene expression was determined in nine leukemia cell lines using the Affymetrix U133 array.

Results: Imatinib was not found to be a substrate for OCT1 in oocytes (P = 0.21), whereas in HEK293 cells IUR was increased by only 1.20-fold relative to control cells (P = 0.002). Furthermore, in 74 cancer patients, the oral clearance of imatinib was not significantly altered in individuals carrying reduced-function variants in SLC22A1 (P = 0.99). Microarray analysis indicated that SLC22A1 was interrelated with gene expression of various transporters, including ABCB1, ABCC4, ABCG2 (negative), and OATP1A2 (positive). Imatinib was confirmed to be a substrate for the three efflux transporters (P < 0.05) as well as for OATP1A2 (P = 0.0001).

Conclusions: This study suggests that SLC22A1 expression is a composite surrogate for expression of various transporters relevant to imatinib IUR. This observation provides a mechanistic explanation for previous studies that have linked SLC22A1 with the antitumor activity of imatinib. Because of its high expression in the intestine, ciliary body, gliomas, and leukemia cells, OATP1A2 may play a key role in imatinib pharmacokinetics-pharmacodynamics.

Imatinib mesylate (Gleevec) is a p.o. administered competitive inhibitor of Bcr-Abl, platelet-derived growth factor receptors (PDGFRα/β), and c-KIT receptor tyrosine kinases (1, 2). This agent has been approved for the treatment of Philadelphia chromosome–positive chronic myeloid leukemia (CML) and also for c-KIT–positive metastatic and unresectable gastrointestinal stromal tumors (3, 4). Substantial interindividual differences in the pharmacokinetic profile of imatinib have been observed in patients with CML or gastrointestinal stromal tumor (5). However, the reasons for this variability are not well-understood (6). Whereas imatinib is well-absorbed after p.o. administration and has an average oral bioavailability of higher than 90% (7), it is still extensively metabolized, with up to 80% of the administered dose being recovered in feces as unchanged drug or metabolites (8). The principal metabolite of imatinib, the N-desmethyl derivative CGP74588, is primarily formed in the liver by CYP3A4, whereas a number of other enzymes such as CYP2D6 are involved in the formation of minor metabolites (9).

It is increasingly recognized that drug disposition is highly dependent on the complex interplay between hepatic drug–metabolizing enzymes and drug transporters (10). However, most studies to date have not considered affinity for transporters as a determinant of the pharmacokinetic profile of imatinib. Although imatinib is a known substrate of the efflux transporters ABCB1 (MDR1, P-glycoprotein; refs. 11, 12) and ABCG2 (BCRP; ref. 13), the mechanisms by which imatinib is taken up into hepatocytes are still unknown. Previous investigations have indicated that a temperature-dependent, active transport mechanism is involved in the intracellular uptake of imatinib in a variety of leukemia cell lines, including CCRF-CEM (14), HL-60 (15), and K562 (16). On the basis of the ability of certain agents, such as verapamil and prazosin, to decrease the uptake of imatinib in a concentration-dependent manner, one of the transporters involved in this process has been identified as the human organic cation transporter OCT1 (14, 16). Because OCT1 is a highly expressed solute carrier in the basolateral membrane of hepatocytes (17), we hypothesized that this transporter facilitates the hepatocellular accumulation of imatinib before metabolism and biliary secretion and, thus, may play an important role in governing drug disposition. The aims of the current study were to assess the interaction of imatinib with OCT1 and a variety of other human uptake transporters using in vitro models, and to
evaluate the pharmacokinetic properties of imatinib in gastrointestinal stromal tumor patients carrying known reduced-function variants of OCT1.

Materials and Methods

Cell lines. The human acute myeloid leukemia cell lines HL-60, Kasumi-1, NB4, ML-2, MV4-11, KG-1, THP-1, L9297, M07e, and the CML cell line K562 were purchased from the American Tissue Culture Collection. All cell lines were cultured in RPMI 1640 containing 10% or 20% fetal bovine serum. M07e cells were supplemented with 10 ng/mL interleukin-3. HEK 293 stable transfectants containing OCT1, OCT2, or OCT3 were obtained from Dr. Heinz Bönisch (University of Bonn, Bonn, Germany; ref. 18); OAT1, OAT2, and OAT3 cells were provided by Dr. Yuichi Sugiyama (University of Tokyo, Tokyo, Japan; ref. 19); OCTN1 and OCTN2 cells were obtained from Dr. Akira Tsuji (Kanazawa University, Kanazawa, Japan; ref. 20). All HEK293 stable transfectants along with pcDNA vector–transfected controls were cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). ABCB1 expression levels in the L-MDR1 cells were maintained by complete medium containing vincristine (640 μmol/L). Saos-2 cells containing pcDNA empty vector, ABCC2, or ABCC4 were also provided by Dr. John Schuetz. These cells were maintained in DMEM containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and G418 sulfate (400-800 μg/mL) at 37°C under 5% CO2 and 95% humidity. The porcine kidney epithelial LLC-PK1 cell line and the L-MDR1 cell lines stably expressing human ABCB1 were kindly provided by Dr. John Schuetz (St. Jude Children’s Research Hospital, Memphis, TN). LLC-PK1 cells were cultured in M199 medium supplied with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). ABCB1 expression levels in the L-MDR1 cells were maintained by complete medium containing vincristine (640 μmol/L). Saos-2 cells containing pcDNA empty vector, ABCC2, or ABCC4 were also provided by Dr. John Schuetz. These cells were maintained in DMEM containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and G418 sulfate (500 μg/mL).

In vitro transport studies. Generally labeled [3H]imatinib was custom made by Moravek Biochemicals, Inc. In all in vitro experiments, radiolabeled imatinib was mixed with unlabeled drug (Novartis) to the desired concentration. The selection of initial test concentration ranges of imatinib in the various (protein free) model systems was based on an average expected unbound drug concentration at steady-state in intestinal stromal tumor patients carrying known reduced-function variants of OCT1. Next, the transport experiment was initiated by adding [3H]imatinib (0.2 μmol/L) to OCT1-expressing cells. For OCTN-expressing cells, a medium was used that contains sodium chloride (125 mmol/L), potassium chloride (4.8 mmol/L), d-glucose (5.6 mmol/L), calcium chloride (1.2 mmol/L), potassium hydrogen phosphate (1.2 mmol/L), magnesium sulfate (1.2 mmol/L), and HEPES (25 mmol/L; refs. 20, 21).

At a predetermined time interval, the experiment was terminated by adding ice-cold PBS after removal of the incubation buffer. After washing with ice-cold PBS, cells were mixed with 900 μL sodium hydroxide (1N) for 20 min. A volume of 25 μL lystate aliquots were used for protein estimation using a bicinchoninic acid protein assay kit (Pierce Biotechnology), and 600 μL lystate were used for analysis of total radioactivity by scintillation counting as described above. The contribution of a given transporter to the intracellular accumulation of imatinib was established by comparing data obtained in HEK293 cells overexpressing the transporter and HEK293 cells transfected with an empty vector.

Transport assays in LLC-PK1 and L-MDR1 cells were carried out as described (22), with minor modifications. Briefly, 2 × 105 cells per well were seeded on a 24.5-mm diameter Transwell plate with a 3.0-μm pore size (Costar). The cells were grown for 3 d in complete medium, with medium changed daily. About 1 to 2 h before start of the experiment, the medium at both the apical and the basal side of the monolayer was replaced with 2 mL fresh medium. The experiment was initiated by replacing the medium at either the apical or basolateral side with 2 mL medium containing [3H]imatinib (1 or 10 μmol/L). The cells were incubated at 37°C, and 50-μL aliquots were taken from each compartment at 1, 2, 3, and 4 h. The appearance of radioactivity in the opposite compartment was measured and presented as the fraction of total radioactivity added at the beginning of the experiment. Calculations were done as described in detail elsewhere (23). In brief, the cumulative amount of imatinib (Q) on the receiver side was initially plotted as a function of time. The steady-state flux J was then estimated from the slope (dQ/dt). The apparent permeability coefficient (Papp) of unidirectional flux for imatinib was estimated by normalizing the flux J (mol/s) against the nominal surface area A (0.33 cm2) and the initial drug concentration in the donor chamber C0 (mol/mL), or Papp = J/(A × C0), expressed in units of centimeter per second. The basolateral/apical ratio equals the Papp value for B-to-A transport divided by the Papp value for A-to-B transport.

For transport studies in Saos-2 cells, cells were seeded in 6-wells plate at density of 5 × 104 per well. At 70% confluence, cells were washed with PBS and replaced with fresh, serum-free medium containing [3H]imatinib (0.1 or 1 μmol/L) for 2 h. At the end of drug incubation, medium was removed and cells were washed twice with ice-cold PBS. The cells were the handled as described above for HEK293 cells.

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Fig. 1. Transport of imatinib by Xenopus laevis oocytes expressing OCT1. Tetraethylammonium (TEA), a known OCT1 substrate, was included as a positive control. Columns, mean of 30 observations and are expressed as percentage of control; bars, SE. The contribution of OCT1 to the intracellular accumulation of imatinib was established by comparing data obtained in oocytes injected with the OCT1 cRNA and water injected control oocytes.
SLC22A1 expression analysis. Total RNA was extracted from cells with TRIzol (Invitrogen), and cDNA was synthesized by the SuperScript First Strand Synthesis kit (Invitrogen). Amplification of SLC22A1 fragments were done using primers published previously (18). PCR reactions were done by using QantiTect SYBR Green PCR Master Mix. The cycling conditions were as follows: 95°C for 15 min, 45 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 30 s. The reaction was run on an ABI 7900HT Fast Real-time PCR System (Applied Biosystems). The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was simultaneously analyzed as a quality control and was used for normalization.

Patient studies. Patients with c-KIT–positive gastrointestinal stromal tumors were treated with p.o. imatinib, given daily at a dose ranging from 100 to 1,000 mg (median dose, 400 mg). Specific inclusion and exclusion criteria have been described previously (6). The study protocol was approved by the Institutional Review Board at the participating institutions, and written informed consent was obtained from each subject according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use/European Union Good Clinical Practice, national, and local regulations. Blood samples were obtained at steady-state at ~28 d after the start of drug administration and analyzed by reversed-phase high performance liquid chromatography with tandem-mass spectrometric detection (24). Pharmacokinetic parameters, including the apparent oral clearance were determined by non-compartmental analysis using the software package WinNonlin version 5.0 (Pharsight). Toxicity and efficacy data were not considered as pharmacodynamic end points in this study because different doses were used in the studied population.

Pharmacogenetic analysis. Genomic deoxyribonucleic acid (DNA) was isolated from plasma of each patient using the UltraSens Virus kit (Qiagen). The selection of two nonsynonymous variants in the SLC22A1 gene at the 286C>T and 1498G>A loci was based on considerations provided elsewhere (25, 26), and included the predicted frequency of the variant alleles in our White patient population and the predicted functional phenotypic change. The OCT1-R61C (SLC22A1 286C>T) and OCT1-G465R (SLC22A1 1498G>A) variants are associated with dramatically decreased function compared with the reference OCT1 protein (25). In this study, OCT1-R61C and OCT1-G465R were genotyped by sequencing exon 1 and exon 9, respectively. The REPLI-g mini/midi kit (Qiagen) was used to amplify genomic DNA based on the manufacturer’s instructions. Primers were designed by using Primer 5.0 version. Primer sequences (5’ to 3’) were gcttagaccccactgactcg (forward) and agacacccacgaactgcac (reverse) for OCT1-R61C and tcctcatggttcctcgtac (forward) and tccctgaagacagaagacaca (reverse) for OCT1-G465R, respectively. PCR reactions were done by using the HotStart Taq Master Mix (Qiagen), following recommended conditions. The efficiency and quality of PCR for all the genes were confirmed by running the PCR products on a 1.5% agarose gel. After clean-up using ExoSAP-IT (USB Corporation), the PCR products were subjected to direct sequencing at the Hartwell Center for Bioinformatics and Biotechnology (St. Jude Children’s Research Hospital). Sequence analysis was done with
Although this value reached statistical significance, the overall contribution of OCT1 to imatinib transport in this model seems limited, in spite of the substantial degree of SLC22A1 overexpression in these cells (Fig. 2B). Interestingly, the mRNA expression was substantially higher in our transfected HEK293 cells relative to that in human K562 cells that were used in a previous study in which imatinib uptake was concluded to be mediated by OCT1 (16). Although the intracellular uptake and retention of \(^{[3]H}\)imatinib was found to be time dependent in the HEK293 cells overexpressing OCT1 (Fig. 2C), the transport was not saturable over a wide concentration range spanning 0.5 to 50 \(\mu\)mol/L (Fig. 2D). This is further suggestive of the lack of a strong interaction of imatinib with OCT1 in this model.

SLC22A1 genotype and imatinib pharmacokinetics. Next, we studied the pharmacokinetics of imatinib in cancer patients with different SLC22A1 genotypes to obtain preliminary information on the \textit{in vivo} relevance of this transporter in the disposition profile of the drug after p.o. administration. In 74 patients with gastrointestinal stromal tumor that were receiving single-agent treatment with p.o. imatinib, 6 subjects were identified that were heterozygous for OCT1-R61C, 1 was

Sequencher Version 4.5 (Gene Codes Corporation). The individuals that carried either one or both of the variants are called the OCT1-variant group, whereas those individuals carrying two copies of the reference sequence are called the OCT1-reference group.

**Microarray analysis.** Total RNA was extracted from nine acute myeloid leukemia cell lines with TRIZol (Invitrogen), and RNA integrity was assessed as described (27). The Affymetrix U133 plus 2.0 GeneChip array (Affymetrix, Inc.) was used to determine expression data according to the manufacturer's protocol. Microarray analysis was done as described (28). The relative expression signals for each gene were calculated using MicroArray Suite version 5.0 (MASS5; Affymetrix). A heat map of interrelationships between the expression of selected solute carriers and ATP-binding cassette (ABC) transporters was generated using the software package Spotfire DecisionSite 7.0 (Spotfire).

**Statistical considerations.** All data are presented as mean values with SE, unless indicated otherwise. Differences in intracellular uptake and retention in the various cell types were evaluated using a Kruskal-Wallis test. Differences in the pharmacokinetic variables of imatinib as a function of OCT1 variants were evaluated using a Mann-Whitney \(U\) test. Two-tailed \(P\) values of \(<0.05\) were considered as statistically significant. All statistical calculations were done using the software package NCSS version 2001 (Number Cruncher Statistical System; J. Hintze).

**Results**

**Transport of imatinib by OCT1.** The interaction of imatinib was initially assessed in \textit{Xenopus laevis} oocytes injected with OCT1 cRNA. In this model, the uptake of the cationic substrate tetraethylammonium was \(~7\)-fold increased in the presence of OCT1 (Fig. 1). However, the intracellular uptake and retention of \(^{[3]H}\)imatinib was only 14.5% higher \((P = 0.21)\) compared with water-injected control cells (Fig. 1). In transfected HEK293 cells, the intracellular uptake and retention of \(^{[3]H}\)imatinib was \(~20\%\) higher in those cells overexpressing OCT1 (Fig. 2A). Although this value reached statistical significance, the overall
homozygous for OCT1-R61C, and another was homozygous for OCT1-G465R. None of the patients carried both variant alleles, consistent with a recent report (29). In this particular cohort of patients, there was no statistically significant difference in the apparent oral clearance of imatinib at steady-state in individuals that had at least one of the reduced-function variants compared with those with the reference allele on both positions (Fig. 3), with median values of 10.37 and 10.41 L/h in the two groups, respectively ($P = 0.99$). The patient homozygote for the R61C variant had an imatinib clearance of 8.47 L/h, whereas the patient homozygote for the G465R variant had a clearance of 4.54 L/h.

**Interaction of imatinib with other solute carriers and efflux transporters.** To identify additional proteins involved in the cellular uptake of imatinib, transport experiments were done in *Xenopus laevis* oocytes or HEK293 cells stably transfected with 10 different cDNAs coding for members of human OATP, OAT, OCT, and novel OCTN families. At a concentration of 0.2 μmol/L, significantly higher uptake rates for [3H]imatinib were found in cells transfected with the human transporters OATP1A2, OATP1B3, and OCTN2 (Fig. 4). Other members of human OATP, OAT, OCT, and OCTN families were incapable of facilitating imatinib transport under these conditions, although they could transport their prototypical substrates (data not shown). Consistent with previous reports (12, 13), we found that imatinib within a clinically relevant concentration range of 0.1 to 10 μmol/L is also recognized by the ABC transporters ABCB1 (Fig. 5A) and ABCG2 (Fig. 5B), with the most efficient transport being observed for ABCB1. Furthermore, a weak but statistically significant interaction was observed between imatinib and ABCC4 (MRP4; Fig. 5B). Interestingly, the mRNA expression of various genes encoding for solute carriers and ABC transporters with a role in imatinib transport, either confirmed here or suspected based on literature data, namely OATP1A2, ABCB1, ABCC3 (30), ABCC4, and ABCG2, was significantly interrelated with expression levels of SLC22A1 in a panel of leukemia cell lines (Fig. 6).

**Discussion**

The present study indicates a minor contribution to the transport of imatinib for OCT1, a solute carrier that is highly expressed in the liver (17), and which had previously been implicated in the intracellular uptake of imatinib on the basis of in vitro studies involving inhibitors of organic cation transporters (14, 16). In overexpressed *Xenopus laevis* oocytes, we found that OCT1-mediated transport of imatinib was essentially absent, whereas in overexpressed HEK293 cells, transport of imatinib was only ~20% increased compared with control cells. Differences in these two experimental systems may be an explanation for the minor disparities in transport characteristics, and this has been described previously for the comparative transport of a number of other agents in amphibian and mammalian cells (31). During the course of preparing this manuscript, Wang et al. (32) showed that OCT1 was capable of transporting imatinib to some extent in the human CML cell line KCL22 after transfection of cells with a pcDNA-OCT1 plasmid. In that study, KCL22 cells with high expression level of OCT1 showed a statistically significant increased influx of imatinib compared with cells transfected with pcDNA vector. Unfortunately, the actual difference in intracellular uptake and retention of imatinib between these two cell types was not provided by authors. However, examination of the data presented suggests that transport of imatinib was ~50% increased in

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**Fig. 6.** Heatmap of interrelationships of transporter expression. Results were obtained for select solute carriers and ABC transporters in nine acute myeloid leukemia cell lines. Red, positive associations; green, negative associations. Arrow, location of the OCT1 gene, SLC22A1.
KCL22-transfected cells compared with control cells. This is not necessarily inconsistent with our current observation but may merely reflect differences in cell context and possible differences between KCL22 cells and HEK293 cells in their respective expression levels of other transporters of relevance to imatinib.

In the current study, we found that patients with gastrointestinal stromal tumor carrying any one of the reduced-function variants OCT1-R61C or OCT1-G465R did not have altered plasma concentrations of imatinib at steady-state compared with patients carrying the reference genotype. In light of the relatively few individuals studied with a variant genotype, however, the currently observed lack of significant relationships between the studied OCT1 variants and the steady-state pharmacokinetics of imatinib has relatively limited statistical power. It is also theoretically possible that additional genetic variants or haplotypes of importance to the pharmacokinetics of imatinib in this population are yet to be discovered. Furthermore, key variants with altered functionality or their frequencies may not be the same in different ethnic populations, as was highlighted recently for studies evaluating the effect of OCT1 variants on the pharmacokinetics of metformin in White (29) and Japanese populations (33). However, this possibility is not supported by the current observation that the normalized expression of SLC22A1 in the transfected HEK293 cells is substantially higher than that in a typical CML cell line. Furthermore, we did not observe statistically significant associations between SLC22A1 expression and the intracellular uptake and retention of imatinib in a panel of leukemia cell lines (data not shown).

An alternative hypothesis that we explored here is that OCT1 might not be by itself causative for the observed relationships of SLC22A1 expression with treatment outcome but, rather, that its expression may be a composite surrogate for expression of various transporters relevant to the intracellular uptake and retention of imatinib. Support for this hypothesis was obtained from a gene expression analysis in leukemia cell lines, in which we found that SLC22A1 is significantly interrelated with various genes previously implicated in imatinib transport.
expression level of OATP1B3 in the liver is much lower than concentration (0.2 μmol/L) that is readily attained in humans (5).

In conclusion, this study indicates that imatinib is a weak substrate for OCT1, and that this transporter by itself is unlikely to contribute substantially to the disposition and activity profiles of imatinib. However, expression of SLC22A1 seems to provide a composite surrogate for the expression of several transporters that are relevant to the intracellular uptake and retention of imatinib. This observation provides a plausible mechanistic explanation for previous studies that have linked the antitumor activity of imatinib with the expression of SLC22A1. Further study is warranted to confirm this observation in clinical samples and cell lines from target disease groups, and to determine the individual and collective contribution of the newly identified transporters OATP1A2 and OCTN2 to the pharmacokinetics and pharmacodynamics of imatinib.

Disclosure of Potential Conflicts of Interest

The authors do not have any conflicts of interest.

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